USER GUIDE

RapidFinder™ Beef ID Kit

Real-time PCR detection of beef DNA in food and feed samples

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For testing of Food and Environmental samples only.

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1 Product information

Product description

Identification of meat species present in food samples is an essential step for verification of origin and traceability of raw materials, as well as for quality control of handling and cleaning processes in production lines. The RapidFinder™ Beef ID Kit enables real-time PCR detection of DNA from beef (*Bos taurus*) that is present in food and feed samples.

The kit includes:

- All reagents necessary for the real-time PCR reaction—specific FAM™-labeled probe and primers for beef mitochondrial DNA, enzyme, and other buffer components.
- An internal positive control (IPC)—VIC®-labeled probe, primers, and template, to monitor for PCR inhibition (included in the reagents).
- A Positive Control, to confirm beef DNA detection.

Unknown samples and control samples are provided by the investigator.

Kit contents and storage

Reagents sufficient for 48 reactions are provided.

Component	Amount	Storage[1]
Beef Master Mix (black pad)	360 µL	–20°C
General Master Mix (white pad)	600 µL	4°C
Positive Control (white cap)	100 µL	–20°C

[1] Refer to the expiration date on the box.

Materials required but not provided

Unless otherwise specified, all materials are available from Life Technologies. MLS: major laboratory supplier.

Item	Source
Instrument and equipment	

Item	Source
Applied Biosystems® real-time PCR thermal cycler and required accessories: • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • 7500 Fast Real-Time PCR System • 7500 Real-Time PCR System	Contact your local sales office.
Adjustable micropipettors (10 µL, 20 µL, 200 µL)	MLS
Benchtop microcentrifuge with adaptors for PCR plates and/or tubes	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Optical reaction plates and covers, *or* optical PCR tubes and caps, as appropriate for your instrument	
For use with the 7500 Real-Time PCR System:	
MicroAmp® Optical Reaction Plate	Cat. no. 4306737
MicroAmp® Optical Adhesive Film	Cat. no. 4311971
MicroAmp®Fast 8-Tube Strip, 0.1 mL (See below for caps.)	Cat. no. 4358293
For use with the StepOne™ Real-Time PCR System:	
MicroAmp® Fast Optical 48-Well Reaction Plate	Cat. no. 4375816
MicroAmp® 48-Well Optical Adhesive Film	Cat. no. 4375323
MicroAmp® Optical 8-Tube Strip (See below for caps.)	Cat. no. 4316567
For use with the StepOnePlus™ Real-Time PCR System or 7500 Fast Real-Time PCR System:	
MicroAmp® Fast Optical 96-Well Reaction Plate, 0.1 mL	Cat. no. 4346907
MicroAmp® Optical Adhesive Film	Cat. no. 4311971
MicroAmp®Fast 8-Tube Strip, 0.1 mL (See below for caps.)	Cat. no. 4358293
For use with all specified real-time PCR systems:	
MicroAmp® Optical 8-Cap Strips	Cat. no. 4323032
Other plastics and consumables	
Aerosol-resistant pipette tips	MLS
1.5-mL nuclease-free microcentrifuge tubes	MLS

Item	Source
Powder-free disposable gloves	MLS
Reagents	
GMO Extraction Kit (recommended for DNA isolation)	Cat. no. 4466336
Nuclease-free Water	Cat. no. AM9938

2 Methods

Input DNA requirements

- Prepare the DNA sample with a method that allows processing of 10–20 g of food sample. The GMO Extraction Kit is recommended.
- Prepare at least one mock-purified sample as an extraction negative control, processed with the same DNA isolation method that is used for test samples.
- Dilute the final DNA sample to 10 ng/µL for the PCR.

Determine the number of reactions and thaw the reagents

1. Plan to include the following reactions:
   - Duplicate reactions for each test sample.
   - Duplicate control reactions.
     - Positive Control (included in the kit).
     - Negative extraction control (mock-purified samples).
     - No-template control reactions; use Nuclease-free Water in place of sample DNA.
2. Thaw all reagents, vortex to mix thoroughly, and place on ice.

Set up the PCR reactions

1. Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
Beef Master Mix (black pad)	7.5 µL
Master Mix General (white pad)	12.5 µL

2. Mix thoroughly by vortexing, and distribute 20 µL to each reaction well or tube.
3. Add 5 µL of DNA sample (10 ng/µL), mock-purified sample (negative extraction control), Nuclease-free Water (no-template control), or Positive Control to the appropriate wells.
4. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom.

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the run using the following parameters:
   - Reaction volume: 25 µL
   - ROX™ passive reference dye: included
   - TaqMan® probe reporter dyes and quenchers:

Target	Reporter	Quencher
Beef	FAM™ dye	MGB
IPC	VIC® dye	MGB

   - Thermal cycler settings:

Setting	Stage 1 Enzyme activation	Stage 2 PCR	
Number of cycles	1 (Hold)	36	
		Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute

2. Load the reactions, run the thermal cycler program and collect real-time amplification data.

Analyze results

The general process for analyzing results is described in this section. The details of data analysis depend on the real-time PCR instrument that you use; refer to the appropriate user guide for instructions on how to analyze your data.

1. View the amplification plots for all reactions to make sure that they appear normal.

2. Use the **Auto** instrument setting for:
   - Baseline
   - FAM™ threshold
   - VIC® threshold

3. Check that the results obtained in all control wells are as expected:

Reaction type	FAM™ channel (Beef)	VIC® channel (IPC)[1]
Positive Control	+	+
Negative extraction control	—	+
No-template control	—	+

[1] In all reactions, the C_T of the IPC should be similar to the C_T of the Positive Control.

For unexpected control results, refer to the troubleshooting section of this guide.

4. Establish the positive cut-off value for the test samples and assign results:
   $C_{T\ (cut\text{-}off)} = C_{T\ (Positive\ Control)} + 3.32$

Sample C_T value	Sample result
$C_T > C_{T\ (cut\text{-}off)}$	Negative
$C_T \leq C_{T\ (cut\text{-}off)}$	Positive[1]

[1] For fresh or minimally processed meat samples, the cut-off values corresponds to approximately 0.01% beef DNA, when the DNA sample concentration is 10 ng/µL.

Interpretation of results

Interpret unknown sample results according to the following table.

FAM™ channel (Beef)	VIC® channel (IPC)	Interpretation
—	+	Beef DNA not detected.
+	+	Beef DNA detected.
—	—	Invalid result. See Chapter 3, "Troubleshooting".
+	—	This result is expected in reactions that have a strong FAM™ signal. See Chapter 3, "Troubleshooting".

Quantitative analysis

For samples with positive results, the percentage of beef DNA with respect to total animal DNA can be quantified using the RapidFinder™ Beef ID Kit in combination with the RapidFinder™ Quant Multi-Meat Set (Cat. no. A24399).

The RapidFinder™ Quant Multi-Meat Set includes the Multi-Meat Standard, a plasmid DNA quantitation standard containing individual species-specific DNA targets and a highly conserved animal-specific mitochondrial genomic region (total animal DNA target). The kit also includes a TaqMan® assay for the total animal DNA target. Both beef and total animal DNA present in each sample can be quantified relative to the Multi-Meat Standard using primers and probe from both kits. For detailed instructions, refer to the *RapidFinder™ Quant Multi-Meat Set User Guide* (Pub. no. MAN0009930).

Note: The Positive Control included in the RapidFinder™ Beef ID Kit is not intended for use as a quantitation standard.

3 Troubleshooting

Observation	Possible cause	Recommended action
In the Positive Control wells, no target-specific and no IPC signals are detected.	PCR amplification failure.	Check that the thermal cycler settings and amplification program are correct.
In the negative extraction control wells, target-specific and IPC signals are detected.	Contamination during the DNA extraction procedure.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. • Check that the DNA extraction protocol was performed correctly. • Take care to avoid contamination during sample homogenization: decontaminate grinding equipment or homogenizer with 10% bleach or DNA*Zap*™ Solutions (Cat. no. AM9890). • Decontaminate benchtop surfaces and other equipment where the DNA extraction process is performed with 10% bleach or DNA*Zap*™ Solutions. • If necessary, use fresh reagents and repeat the DNA extraction.
In the no-template control wells, target-specific and IPC signals are detected.	Contamination of the PCR.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. • Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach or DNA*Zap*™ Solutions (Cat. no. AM9890). • Use fresh reagents and repeat the PCR. • Set up the Positive Control PCR reactions last to avoid cross-contamination.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In unknown wells, no IPC or target-specific signal is detected.	Excess sample DNA in the PCR; the recommended maximum is 250 ng.	Repeat the PCR with the correct amount of DNA.
	PCR inhibitors in the sample DNA.	Repeat the DNA extraction. If the problem persists, contact Technical Support.

A Kit sensitivity and specificity

The detection limit was calculated with standard samples consisting of mixtures of raw beef meat and other species. The RapidFinder™ Beef ID Kit can detect blends with as little as 0.01% (w/w) of beef meat. The limit of detection in processed samples varies depending upon the composition and food processing.

The kit specificity was tested by comparison of the probe and primer sequences with the NCBI database, and it was also experimentally tested on a collection of reference DNAs, with the following results.

Meat species	Result
Sheep	Not detected
Goat	Not detected
Horse	Not detected
Beef	Detected
Pork	Not detected
Buffalo	Not detected
Deer	Not detected
Chicken	Not detected
Turkey	Not detected
Duck	Not detected
Ostrich	Not detected
Goose	Not detected
Human	Not detected

B Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. no. AM9890).

For additional information, refer to ISO 22174 (2005).

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps for PCR tubes come in strips of 8 or 12.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Food Safety support

Website: **www.lifetechnologies.com/foodsafety**

Support email: **foodsafety@lifetech.com**

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **www.lifetechnologies.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Limited product warranty

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